RESEARCH NOTE

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Comparison of PCR detection of *mecA* with agar dilution and Etest for oxacillin susceptibility testing in clinical isolates of coagulase-negative staphylococci

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ABSTRACT

Oxacillin-resistant staphylococci are heterogeneous in their expression of resistance to β-lactam antibiotics. Different recommendations regarding screening methods for routine use have been published. In this study, the susceptibility to oxacillin of 232 coagulase-negative staphylococci (CoNS) was determined by agar dilution, Etest and presence of the *mecA* gene. When an oxacillin resistance breakpoint of ≥0.5 mg/L was used, the sensitivity and specificity for agar dilution were 97.6% and 100%, and those for Etest were 100% and 95.4%. The current National Committee for Clinical Laboratory Standards oxacillin breakpoint recommendation will categorise accurately the CoNS species encountered commonly.

Keywords Agar dilution, coagulase-negative staphylococci, Etest, oxacillin resistance, PCR, susceptibility testing

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Coagulase-negative staphylococci (CoNS) are a significant cause of bacteraemia and other hospital-acquired infections, especially in immunocompromised individuals and patients with prosthetic implants [1–3]. Many CoNS are resistant to penicillin and oxacillin because of production of β-lactamase and penicillin-binding protein 2a (PBP2a), respectively [4]. Production of PBP2a in staphylococci is encoded by the *mecA* gene. PBP2a has been found in a variety of CoNS, but expression of *mecA* is different in CoNS and *Staphylococcus aureus*. Several groups of investigators have described DNA probes [5–7] and PCR assays [8–16] for detection of oxacillin resistance in staphylococci. Based on the results of some of these studies, the National Committee for Clinical Laboratory Standards (NCCLS) has redefined the breakpoints for oxacillin susceptibility in CoNS [17]. In the present study, PCR detection of the *mecA* gene was compared with Etest and agar dilution based on the new NCCLS breakpoint for identification of oxacillin resistance in clinical isolates of CoNS.

Clinically significant isolates ($n = 180$) were collected from 13 Norwegian clinical microbiological laboratories over a period of 22 months. Preliminary identification in the local laboratory was on the basis of colony morphology, a positive catalase test, and a negative agglutination test or a negative DNase test. CoNS blood culture isolates that had been collected and stored at Haukeland University Hospital for the previous 2 years were also included in the study ($n = 52$). The 232 isolates were from the following sources: blood culture (160 isolates), venous and arterial catheters (32), surgical wounds (30), tracheal tubes (four), spinal fluid (two), peritoneal fluid (two), pleural fluid (one) and eye secretion (one). All isolates were identified to the species level by API Staph 32 ID (bioMérieux, Marcy l'Etoile, France) with an automated reader at Haukeland University Hospital.

Etests (AB Biodisk, Solna, Sweden) were performed following the recommendations of the Norwegian Working Group on Antibiotics [18]. Agar dilution was performed with Mueller–Hinton agar (Mast, Bootle, UK) supplemented with NaCl 2% w/v, an inoculum of 0.5×

Table 1. Correlation between the oxacillin MIC (agar dilution) for different CoNS species and the presence of the *mecA* gene

Staphylococcus spp.	*mecA*	No. of isolates tested	Number with MIC values (mg/L): ≤ 0.13	0.25	0.5	1	2	4	≥ 6
S. epidermidis	+	105		2	13	39	21	8	22
	–	71	68	3					
S. hominis	+	2		1					1
	–	12	11	1					
S. haemolyticus	+	12							12
	–	0							
Other CoNS	+	3			1	1			1
	–	6	4	2					
S. warnerii	+	1				1			
	–	6	6						
S. capitis	+	1						1	
	–	5	5						
S. lugdunensis	+	0							
	–	2	2						
S. chromogenes	+	0							
	–	2	2						
S. schleiferi	+	0							
	–	1	1						
S. saprophyticus	+	0							
	–	1	1						
S. xylosus	+	1			1				
	–	0							
S. simulans	+	0							
	–	1		1					
Total		232							

McFarland standard, and incubation for 24 h at 34 ± 1 °C. *MecA* PCR was performed as described by Predari *et al.* [9] at the Telelab laboratory, with the following modification in the lysis procedure. The pellet was resuspended in 100 μL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) with 10 μL lysostaphin (15 U/mL). Following incubation at 37 °C for 15 min, 10 μL sodium dodecyl sulphate 1% w/v and 10 μL proteinase K (25 mg/mL in 50 mM Tris-HCl, pH 8.0) were added, followed by incubation for a further 15 min at room temperature.

In total, 125 (53.8%) of the 232 CoNS isolates were positive for the *mecA* gene by PCR. Table 1 shows the correlation between agar dilution MICs and the presence of the *mecA* gene within the different CoNS species identified. The current breakpoint for oxacillin resistance separated the resistant (*mecA*-positive) and susceptible (*mecA*-negative) populations with satisfactory precision. Only three *mecA*-positive isolates had MICs below the actual breakpoint, with values of 0.25 mg/L. Table 2 shows the correlation between *mecA* PCR and susceptibility tests performed by agar dilution and Etest with an oxacillin breakpoint of ≥0.5 mg/L. The sensitivity for agar dilution was 97.6%, and that for Etest was 100%. The specificity for agar dilution was 100%, and that for Etest was 95.4%. If the oxacillin resistance breakpoint had been lowered to ≥0.25 mg/L, the sensitivity for agar dilution would have reached 100%, but the specificity would have decreased to 92.6%. The values that decreased the specificity for the oxacillin Etest were in the range 0.5–6.0 mg/L.

CoNS are the pathogens isolated most commonly from bloodstream infections in intensive care unit patients [3]. Of 32 CoNS species recognised, only half are seen in clinical specimens. Among CoNS, *S. epidermidis* is generally the most common species [1]. In total, 54% of the isolates studied were resistant to oxacillin, as determined by PCR detection of the *mecA* gene. This value is lower than in other published reports, which have detected resistance rates of 70–80% [2]. In the present study, the sensitivity and specificity of agar dilution were satisfactory when the current oxacillin MIC breakpoint of ≥0.5 mg/L was used. Two *S. epidermidis* and one *S. hominis* isolate would have been reported as falsely oxacillin-

Table 2. Correlation between oxacillin agar dilution with a resistance breakpoint of ≥0.5 mg/L, Etest, and the presence of the *mecA* gene

	Agar dilution[a]			Etest[b]		
	+	–		+	–	
mecA						
+	122	3	125	125	0	125
–	0	107	107	6	101	107
	122	110	232	131	101	232

[a]Sensitivity 97.6%, specificity 100%.
[b]Sensitivity 100%, specificity 95.4%.

susceptible, but full correlation was achieved among all the other species.

The findings confirm that, among the species encountered commonly, the NCCLS oxacillin breakpoint of ≥0.5 mg/L results in correct classification of CoNS as oxacillin-resistant or -susceptible in most cases.

Etests correlated well with PCR for detection of oxacillin resistance in the CoNS isolates, but six of 102 *mecA*-negative isolates were classified falsely as resistant. Huang *et al.* [19] found discrepancies between Etest and agar dilution when testing CoNS. Etest results were skewed towards MICs of oxacillin higher than these given by agar dilution. Both Weller *et al.* [10] and Frebourg *et al.* [20] found major discrepancies when comparing Etest with PCR, but the results would have been improved if the resistance breakpoint had been lowered from 2 to 0.5 mg/L.

Six reports have either proposed revised criteria for oxacillin susceptibility [2,8] or confirmed [11,14–16] that the new NCCLS oxacillin breakpoint [17] classifies most of the commonly encountered species of CoNS correctly as oxacillin-susceptible or -resistant, but among the more uncommon CoNS species, the oxacillin agar dilution test can lead to the incorrect classification of *mecA*-negative isolates as oxacillin-resistant. There are, however, small discrepancies among these studies, which may be caused by differences between the isolates analysed or problems with the difficult CoNS speciations [14]. Overall, it seems that the current NCCLS oxacillin breakpoint recommendation will categorise accurately the CoNS species encountered commonly. However, it is recommended that isolates of CoNS should be identified phenotypically and oxacillin resistance should be confirmed with PCR for isolates from patients with serious infections when the choice of antibiotic is crucial [14,15].

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RESEARCH NOTE

Management of long-term catheter-related *Brevibacterium* bacteraemia

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ABSTRACT

Brevibacterium has been reported as a rare cause of implanted-device infection. In two cases of recurrent *Brevibacterium casei* bacteraemia associated with infection of surgically implanted intravascular devices, relapse occurred 2 and 5 months, respectively, after completion of therapy with vancomycin via the infected catheter. A second intravenous antibiotic therapy course by the antibiotic-lock technique led to bacteriological cure in one patient. Molecular typing results demonstrated that the recurrent bacteraemia was caused by the same strain. Implanted-device removal may be necessary, in addition to appropriate antibiotics, for successful management of such infections.

Keywords Bacteraemia, *Brevibacterium*, catheter infections, management

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Brevibacterium spp. are non-motile, non-spore forming, catalase-positive, aerobic Gram-positive bacilli. They are part of the normal flora of the skin and adjacent structures, but have been reported only rarely as causes of human infection, including endocarditis [1], continuous ambulatory peritoneal dialysis peritonitis [2] and bacteraemia in compromised hosts with long-term intravenous devices [3–8]. Other habitats include raw milk, cheese and animal sources. The genus *Brevibacterium* contains at least eight species (*B. linens*, *B. casei*, *B. epidermidis*, *B. iodinum*, *B. mcbrellneri*, *B. otitidis*, *B. avium* and *B. paucivorans* [9]). Identification to the genus level is achieved easily with commercial identification panels, but species identification requires additional biochemical tests, fatty acid analysis or genotyping.

Management of catheter-related bloodstream infection varies according to the type of catheter involved and the causative pathogen. Removal of the infected catheter in case of complicated bacteraemia is consensual [10]. However, for tunnelled or implanted catheter-related infection, other factors, including the type of pathogen, local signs of infection, and the severity of infection are involved in the decision whether or not to remove the catheter. Conservative intravenous antibiotic therapy associated with an antibiotic-lock is recommended only for uncomplicated bacteraemia caused by organisms of low virulence [6].

Table 1 summarises the clinical features of two unusual cases of recurrent bacteraemia caused by *B. casei* that were associated with infected long-term intravascular access. The first patient was a 43-year-old woman, who suffered from Crohn's disease and developed pelvic entero-cutaneous fistulisations. This required total colectomy with discharge jejunostomy, which was complicated by chronic entero-cutaneous fistulae. Since this time, she received total parenteral nutrition through a totally implantable intravenous port (Port-A-Cath®). Five years later, she presented with recurrent pyrexia. The second patient was a 31-year-old man with an implanted Hickman catheter who became febrile (38.5 °C) during haemodialysis. He had no specific symptoms and physical examination was normal, with no sign of catheter infection and no noticeable inflammatory syndrome. In both cases, blood culture yielded *B. casei*, and intravenous therapy with vancomycin was initiated. Both patients